Appendix A

GENERAL PROGRAM PRIZE PAPERS

Monday, September 27, 1999
11:25 A.M.

O-001

Spindle Observation and Its Relationship with Fertilization after ICSI in Living Human Oocytes. W. H. Wang, R. J. Hackett, L. Meng, D. L. Keefe. Department of Obstetrics and Gynecology, Women & Infants' Hospital of Rhode Island, Brown University School of Medicine, Providence RI.

Objective: The LC polscope allows non-destructive imaging of macromolecular structures within cells based on their birefringence. The spindle in oocytes is highly birefringent and its integrity essential for normal chromosome segregation during meiosis. Viewing the spindle in living human oocytes is valuable to study spindle dynamics and oocyte viability. In this study our objectives were 1) to image spindles in normal and aged human oocytes and 2) to examine the relationship between spindle structure and fertilization after intracytoplasm sperm injection (ICSI).

Design: The LC polscope (CRI, Cambridge, MA) was used to examine spindles in living oocytes before ICSI or in aged, unfertilized oocytes 1–4 days after ICSI or IVF. Oocytes with or without spindles were separated after ICSI to examine subsequent fertilization. Aged oocytes were further examined by immunocytochemical staining and confocal microscopy.

Materials and Methods: 1) Oocytes were recovered from patients in an academic IVF clinic. After retrieval, cumulus-oocyte complexes were cultured for 5–6 h in HTF containing 10% synthetic serum substitute. Cumulus was removed from oocytes before examination. All oocytes with visible polar body (Pb) in the perivitelline space were examined before ICSI and the temperature was kept at 37°C during imaging and ICSI. After ICSI, oocytes with or without visible spindles under the polscope were cultured separately. Fertilization rates were examined 14–16 h after ICSI. Size of spindle and distance between spindle and Pb were measured with a computerized image analysis system. 2) Discarded oocytes (1–4 day old) that remained unfertilized after IVF or ICSI were examined by Polscope, and then stained by anti-tubulin antibody and examined by laser confocal microscopy to compare the two imaging modalities.

Results: 1) 162 oocytes from 16 women were examined and spindles were visible in 59.9% of oocytes. The size of spindles and distance between spindles and Pb's were 15.24 +/− 2.33 μm and 36.42 +/− 18.19 μm, respectively. The spindle was close to Pb in most day 1 oocytes. After ICSI, more (63.9%; $P < .05$) oocytes with spindles were fertilized than oocytes without spindles (46.2%). Most fertilized oocytes (88%) had 2 pronuclei, with no difference being observed between oocytes with or without spindle. 2) only 2 oocytes on day 1 had intact spindles while in 30 oocytes the spindles had depolymerized with only a few microtubules remaining around the chromosomes or dispersed in the cytoplasm. In most aged oocytes, chromosomes were scattered throughout the cytoplasm. Confocal images of immunostained spindles were almost identical to polscope images of spindle birefringence.

Conclusions: This study indicates that spindles in living human oocytes can be imaged with the LC Polscope. This technology can be used to monitor spindle position in human ICSI and to study spindle dynamics in living human and other mammalian oocytes. The presence of a birefringent spindle predicts increased fertilization rate after ICSI. The LC Polscope may help select structurally normal oocytes for selective rescue ICSI.